UNIT-I

Laboratory Animals

Points to be covered in this topic

❑ Laboratory Animals

- Study of CPCSEA and OECD guidelines for maintenance,
- breeding and conduct of experiments on laboratory animals,

❖ Common lab animals:

- Description and applications of different species and strains of animals.
- Popular transgenic and mutant animals.
- Techniques for collection of blood and common routes of drug administration in laboratory animals,
- Techniques of blood collection and euthanasia.

Study of CPCSEA and OECD guidelines for maintenance

CPCSEA GUIDELINES

❑ Introduction

- **Background and Objectives:**

The motto of **Prevention of Cruelty to Animals (PCA) Act 1960** as **amended in 1982** is to prevent infliction of **unnecessary pain or suffering on animals.** The Central Government has constituted a **Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA),** which is duty bound to take all such measures as may be necessary to ensure that animals are not subjected to unnecessary pain or suffering before, during or after the performance of experiments on them. For this purpose, the Government has made **"Breeding of and Experiments on Animals (Control and Supervision) Rules, 1998"** as amended during **2001** and **2006** to regulate the experimentation on animals.

The following guidelines are motioned in the CPCSEA, 2006:

The main functions of CPCSEA are:

- Registration of establishments **conducting animal experimentation** or **breeding of animals** for this purpose.
- Selection and assignment of nominees for the **Institutional Animal Ethics Committees (IAEC)** of the registered establishments.
- **Approval of Animal House Facilities** on the basis of reports of inspections conducted by CPCSEA.
- **Permission for conducting experiments** involving use of animals.
- Recommendation for **import of animals** for use in experiments. Action against establishments in case of established violation of any legal norm/stipulation.
- Conduct of **Training Programmes** for the Nominees of CPCSEA.
- Conduct/Support of Conference/Workshop on Animal Ethics.

Requirement of form in CPCSEA supervision

- **Application for registration** of breeder/establishment/educational institution (Form A).
- **Application for permission** for animal experiments (Form B).
- **Record of animals bred/acquired** and record of animals acquired and experiments performed (Form C and D).
- **Record of animals sold** to the establishment by the traders (Form-E).

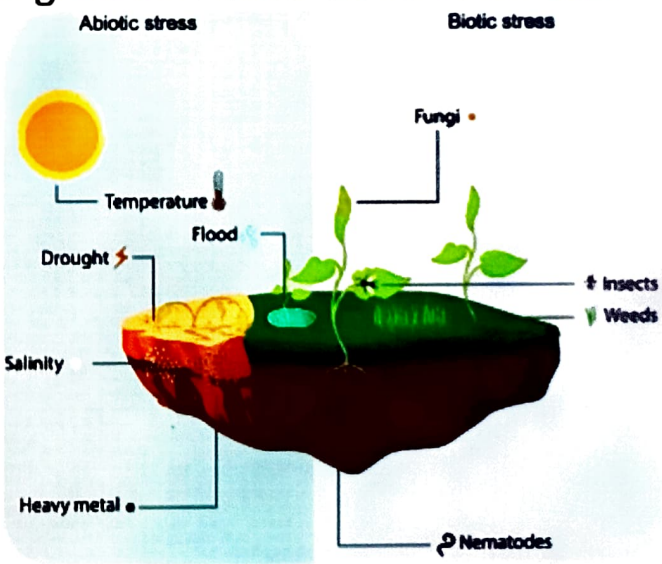
OECD GUIDELINES

Testing guidelines are developed for a variety of reasons: **to allow results of various test substances** or species to be easily compared, to **encourage the use of certain protocols** so that testing need not be repeated, and to facilitate the work of those who design and **carry out tests.** Many organizations have developed testing guidelines, such as **OECD Guidelines, FDA Guidelines** and **Pesticide Assessment Guidelines** for involving whole animal testing.

The Organization for Economic Cooperation and Development (OECD) guidelines have wide acceptance in the United States and abroad because of the **Mutual Acceptance of Data Decision Under the terms of this decision**, member countries of OECD must accept data generated in other countries if done so according to these guidelines. The **OECD was officially born on 30th September 1961.** Some animal tests contained in the guidelines are listed below with the OECD guideline numbers for reference:

1. **Effects on Biotic Systems**

- 202 Daphnia, **acute immobilization test and reproduction test.**
- 203 **Fish, acute toxicity test.**
- 204 Fish, **prolonged toxicity test:** 14 days study.
- 205 Avian **dietary toxicity test.**
- 206 Avian **reproduction test.**



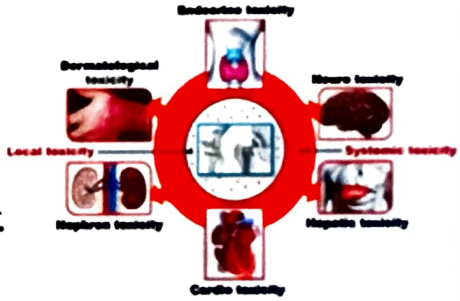
2. Degradation and Accumulation

- 305A Bioaccumulation: **Sequential Static Fish Test.**
- 305B Bioaccumulation: **Semi-static Fish Test.**
- 305C Bioaccumulation: Test for the **Degree of Bio-concentration** in Fish.
- 305D Bioaccumulation: **Static Fish Test.**
- 305E Bioaccumulation: **Flow-through Fish Test.**

3. Health Effects

I. Short Term Toxicology

- 401 Acute **oral toxicity.**
- 402 Acute **dermal toxicity.**
- 403 Acute **inhalation toxicity.**
- 404 Acute **dermal irritation/corrosion.**
- 405 Acute **eye irritation/corrosion.**
- 406 **Skin sensitization.**
- 407 Repeated **dose oral toxicity** rodent: 14/28 days.
- 408 Sub **chronic oral toxicity** rodent: 90 days
- 409 Sub **chronic oral toxicity non rodent**: 90 days.
- 410 Repeated **dose dermal toxicity**: 14/28 days.
- 411 Sub **chronic dermal toxicity:** 90 day.
- 412 Repeated **dose inhalation toxicity:** 14/28 days.
- 413 Sub **chronic inhalation toxicity:** 90 days.
- 414 **Teratogenicity.**
- 415 **One-generation** reproduction toxicity study.
- 416 **Two-generation** reproduction toxicity study.
- 417 **Toxicokinetics**
- 418 Acute delayed **neurotoxicity of organophosphorus** substances.
- 419 Sub chronic **delayed neurotoxicity of organophosphorus** substances: 90 days.



II. Long Term Toxicology

- 451 **Carcinogenicity studies.**
- 452 **Chronic toxicity studies.**
- 453 **Combined chronic** toxicity/carcinogenicity studies.

Breeding and conduct of experiments on laboratory animals

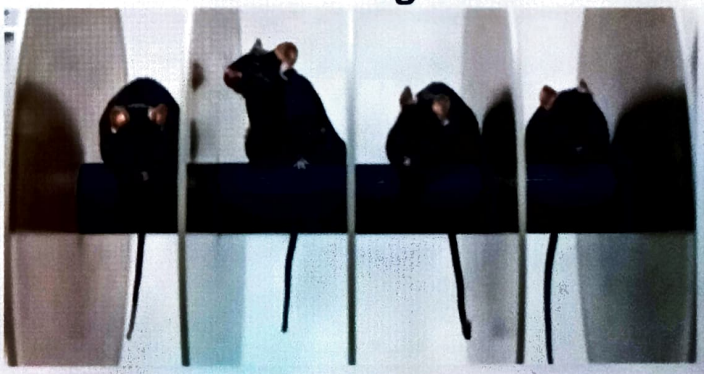
- Experimentation on animals in course of medical research and education is covered by provisions of the **Prevention of Cruelty to Animals Act, 1960 and Breeding of and Experiments on Animals (Control and Supervision) Rules of 1998, 2001 and 2006 framed under the Act.**

- These are enforced by the **Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA),** a statutory body under the **Prevention of Cruelty to Animals Act, 1960.**

- Under these provisions, the concerned establishments are required to get themselves **registered with CPCSEA, form IAEC,** get their Animal House Facilities inspected, and also get specific projects for research cleared by CPCSEA before commencing the research on animals.

- Further, breeding and trade of animals for such experimentation are also regulated under these Rules. In an **amendment bought out in 2006** in the Rules for **Breeding of and Experiments on Animals** (Control and Supervision), powers to permit experiments on small animals were given to Institutional Animal Ethics Committee (IAEC) of the establishments.

- Only proposals for conducting experiments on large animals are required to be **sent to CPCSEA for approval.** Accordingly, it is important that all the IAEC members are fully aware of the extant rules and guidelines.



The experimental animals regulated by the Animal Protection Act dated 21st August, 1997 (Law Reports no, 111, item 724), the legal issues are:

- Research or experimental protocols tests on laboratory animals may be performed only in **scientific institutions approved by CPCSEA** under the guidance and supervision of IACE.
- Experiments on animals are performed only when they are **mandatory for scientific research, university education or the protection** of the health of people or animals if these objectives cannot be achieved otherwise as no alternative methods exist.
- The research protocol must be approved by the **Local Ethics Committee (IAEC)** supervised by the **National Ethics Committee (CPCSEA)**
- The husbandry of laboratory animals must be licensed by the Ministry of Agriculture and Food Economy and animal house should ensure the conditions appropriate for animal species.
- Experimental **protocol associated with pain** must to be performed in **general or local anaesthesia** only once on one individual, unless the nature of the experiment requires its repetition on the same animal. The experiments may be performed **without anaesthesia** only in exceptional cases, when it is obligatory from the scientific point of examination.

Some terms used in experimental laboratory are as follows:

Animal experiment: Utilization of **animals for education, testing, research, manufacture of pharmaceutical products, biological products** or other scientific purposes.

Facilities: Facilities and equipments used to perform animal experiments.
Laboratory animal: **Animal of Pisces, reptilian, amphibians, avian, and mammalian species used as experimental animal.**

Institution: Organization (university, institute, independent administrative body, company, etc.) where animal experiments are performed.

Director of institution: Person with overall responsibility in the institution for proper and safe conduct of the animal experiments (dean, director of an institution, principal of a school, chairperson of the board of directors, president, head of an institute, etc.)

Animal experiment protocol: Protocol drafted beforehand for the conduct of an animal experiment.

Researchers: Persons who are performing the animal experiment.

Principal investigator: The researcher who is in charge of all duties related to the animal experiment protocol or research.

Manager: Person in charge of the laboratory animals and facilities under the director of the institution (head of the animal experimentation facilities, department head, animals house in charge etc.).

Laboratory animal manager: The laboratory animal manager assists the manager and is in charge of management of the laboratory animals.

Animal technician: Person in charge of care and management of laboratory animals under the laboratory animal manager or researcher.

Policies: Fundamental guidelines and basic policies specified by government agencies related to animal experiments and "Guidelines for Proper Conduct of Animal Experiments" (these Guidelines) specified by the Science Council of Japan.

Regulations: In house regulations of research institutions specified for the **proper conduct of animal experiments** and the **proper care and management of laboratory animals** based on related laws and ordinances and the policies

Common Lab animals

ALBINO MOUSE		
Zoological Name	**Mus musculus**	 **Albino swiss mouse**
Other strains	**Swiss mice Laca, C-57.**	
Age	**1-2 years**	
Estrous or oestrous cycle	**4-5 days**	
Mating age	**6-8 weeks**	
Gestation period	**19-21 days**	
Body weight (adult)	**23-30 g**	
Feed	**Cracked food, shark liver oil, yeast powder, pellets, sesame oil, fish, some other supplements etc.**	
Use	**Acute toxicity study, assay of insulin, cancer and genetics research, screening of chemotherapeutic and teratogenic agents, study of analgesics, behavioral study etc. Isolated preparation: vas deferns, ileum, trachea, fundus etc.**	

ALBINO RAT

Zoological Name	Rattus norvegius
Other strains	rat, Wistar rat Porton, Biobreeding rat, Long evans rat RCS rat, Zucker rat, Shaking rat Kawasaki .
Age	2-3 years
Estrous or oestrous cycle	4-5 days
Mating age	10-12 weeks
Gestation period	21-23 days
Body weight (adult)	180-260 g
Feed	Cracked food, shark liver oil, yeast powder, pellets, sesame oil, fish, some other supplements etc.
Use	Toxicity study, assay of hormones, cancer and genetics research, screening of chemotherapeutic and teratogenic agents, study of analgesics, behavioral study, anti ulcer, antihypertensive, anti-diabetics, liver physiological studies etc. Isolated preparation. vas deferens, ileum, trachea, fundus, uterus, colon, evaluation of psychopharmacological agents etc.

DOMESTIC RABBIT

Zoological Name	Oryctolagus cuniculus
Other strains	New Zealand white strains.
Age	4-5 years
Estrous or oestrous cycle	4-5 days
Mating age	6-9 months
Gestation period	28-31 days
Body weight (adult)	2-3 kg
Feed	Lucerne grass, carrot, bengal gram, wheat bran etc.
Use	Pyrogen testing, anti diabetic activity, bioassay of insulin, embryo toxic study, capillary permeability study.

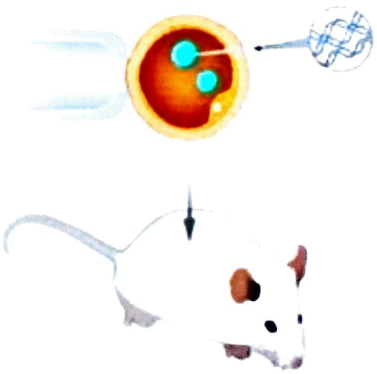
HAMSTER

Zoological Name	Mesocricetus ouratus
Age	2-3 years
Estrous or oestrous cycle	4 days
Mating age	6-8 week
Gestation period	15-18 days
Body weight (adult)	85-150 g
Feed	Soya bean meal, carrot, corn starch etc.
Use	Immunology, virology, diabetic, cytological studies, genetic, tissue culture and radiation study etc.

Popular transgenic and mutant animals

There are five main categories of transgenic animals.

Disease models	Include animals such as Alzheimer's mouse, AIDS mouse, and OncoMouse that are engineered to mimic some aspects of a human disease. These animals provide models for investigating disease mechanisms and potential cures.
Transpharmer	Include animals engineered to express protein drugs or antibiotics in their milk. These animals provide a convenient source for new medications with no animal sacrifice. This category includes such models as Baby Herman (the world's first transgenic cow, whose female offspring provide the first successful bovine transpharmer) and the Genzyme goats.
Xenotransplanter	Those are engineered to produce organs compatible with humans.
Transgenic food sources	That includes animals like Super pig and Super fish that are engineered to be larger than normal.
Transgenic include scientific models	That teach us something new about a specific protein's function in vivo (Rudolph and Mohler, 1999).



Some of the type of genetically modified animals are mentioned below (Edgar and Sibille):

Organism	Synonyms	Definition
Transgenic organism	Mutant	Traditionally, an organism with the addition of foreign DNA, whether from the same species or a different one.
Knockout		The removal or complete disruption of a specific gene in an animal from the blastocyst stage through adulthood.
Knockin		The introduction of a mutated version of a specific gene in place of the wild type version.
Conditional knockout	Site-specific knockout, region-specific knockout	The removal or complete disruption of a specific gene in a manner that controls the cell types and brain region or site where the disruption occurs.
Inducible knockout	Time-specific knockout, temporal knockout.	The use of a system where the experimenter controls the timing of gene removal.
RNAi (RNA interference)	siRNA (small interfering RNA)	An endogenous system where short sequences of double stranded RNA molecules induce the cleavage of matching mRNA resulting in down regulation of a particular gene.
Chimera	Mosaic	An animal where individual cells contain genetic material from only one of two potential lineages.
Forward Genetics / Reverse Genetics	Forward genetics Random mutagenesis	Phenotype introduced by a chemical mutagenesis or mutation by irradiation (e.g. phenotype → genotype).
Phenotype/Genotype		Genotype is the specific genetic constitution of an organism including the gene allelic makeup. Phenotype is the physical trait or characteristic arising from the genotype.
Pseudopregnant		A hormonal state similar to pregnancy that is induced in mice by mating a female with a vasectomized male. In this state, the uterus is receptive to an implanted embryo.

Transgenic History:

- The **first chimeric mice were produced during the 1970s** (Brinster, 1990). Chimera is an organism that contains a mixture of genetically different cells.
- The cells of **two different embryos** of **different strains of mice** were combined together at an early stage of development to form a **single embryo that later developed into a chimeric adult.** The adult was chimeric because it **exhibited characteristics of each strain of the two original embryos.** The combined contributions of developmental biology and genetic engineering permitted a quick development of the techniques **used for the creation of transgenic animals.**
- **DNA microinjection** was the **first transgenic technique to prove successful in mammals.** It was first applied to mice and then to various other species such as **rats, rabbits, sheep, goats, cows, pigs, birds, chickens, and even fish.** Two other techniques that were later developed were called **retrovirus-mediated transgenesis** and **embryonic stem cell-mediated gene transfer.**
- The term **"transgenic"** was first used by **J.W. Gordon** and **F.H. Ruddle** in 1981. In 1980, Gordon and Ruddle produced the **first transgenic mouse through pronuclear injection of a fertilized egg cell.**
- Since then, there has been rapid development in the **use of genetically engineered animals as investigators** have found an increasing number of applications for the technology. Some examples of transgenic uses are in the **pharmaceutical, therapeutic, agricultural,** and **medical industries.**